Magneto-hydrodynamic and Microwave Measurements for Label-free Detection and Differentiation of Bacterial Pathogens

A synopsis of the proposed thesis to be submitted for the degree of

DOCTOR OF PHILOSOPHY
in
PHYSICS
by

Zeeshan

Under the Supervision of:
Prof. K. Soami Daya
(Department of Physics and Computer Science)
Dayalbagh Educational Institute, Agra

Co-Supervisor
Prof. Evangelyn C. Alocilja
(Department of Biosystems and Agricultural Engineering)
Michigan State University, U.S.A

Department of Physics and Computer Science

DAYALBAGH EDUCATIONAL INSTITUTE
(DEEMED UNIVERSITY)
DAYALBAGH, AGRA (UP) – 282005

(MARCH–2019)

INTRODUCTION

Bacterial pathogens, their detection and challenges

Despite having an annual budget of ₹52,530 crores for improving the epidemiological aspects of living standards in India, disease management and control of mortality is still a national concern. According to National Health Profile (a 2018 report from Ministry of Health and Family Welfare, India), of all deaths occurring due to diseases, 70% are because of one or the other type of infection [1-4]. Moreover, as WHO reports, situation in other parts of the globe are not much different [5]. In past two years infectious diseases (bloody diarrhoea, skin infections, organ dysfunctions and sepsis *etc*.) have claimed 8 million lives worldwide with bacterial pathogens being the prime cause [6].

Generic symptoms of diseases, negligence of patients, doing self-medication and use of broad-spectrum antibiotics escalates the risk of 'drug resistance' [7,8]. For efficient management and control of diseases, timely and specific treatment is essential, which depends on accuracy and rapidity of diagnosis. Requirement for rapid detection in clinical samples is apparent to avoid delay in course of treatment. While, food industry yearns for rapid and sensitive methods of microbial testing, to immediately detect pathogens in raw materials, finished product and to monitor the process control [11].

A variety of methods are available for detection of pathogens, among which traditional microbiological methods like cell culturing and biochemical testing are most reliable, however, sterility and tedious pre-processing with long detection time does not meet the purpose of rapid diagnosis [9]. Unlike microbiological methods, DNA based assaying (ELISA, PCR and nucleic acid sequencing) is comparitively faster (gives result within 24 hours) but requires extensive processing,; that includes cell-lyses, DNA extraction, amplification and purification, all to be done through extremely expensive and high-precision instruments [10]. In contrast to core microbiological and DNA-based techniques, methods like quartz crystal microbalance (QCM), microcantelever chips and Bio-FETs etc. are provenly rapid, but, their specificity depends on availability of antibody and other molecules for immobilizing antigen on the surface of the detector [10].

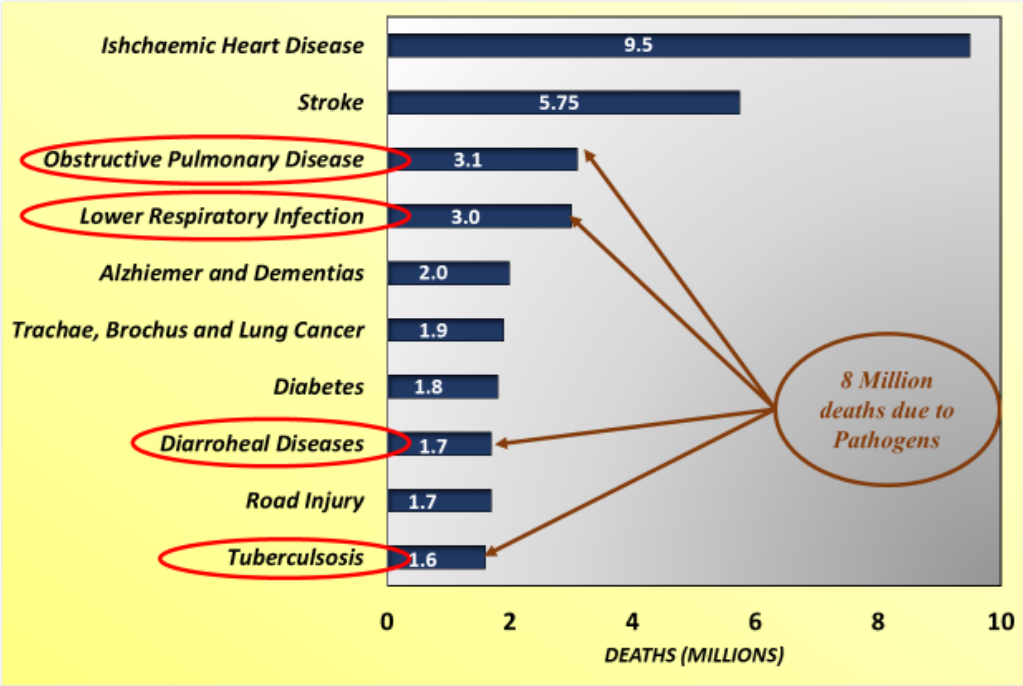


Fig.1 Top 10 causes of deaths worldwide (Global health Observatory Data (2018)) [38]. The red circles highlight deaths caused by pathogenic infections.

Being aware of the rising challenges in detection of pathogens in clinical and industrial samples, Mandal *et. al.* postulated, that systems for the detection of pathogens or the biosensors should be (i) efficient in detecting desired specific micro-organism, (ii) sensitive towards very small number of pathogens (iii) having multiplexed detection of different bacteria on the same sensor and (iv) giving a quantitative estimate for number of micro-organisms. Currently, no biosensor meets the requirements in full [11].

MOTIVATION

Rapid diagnosis of blood sugar level through commercialised biosensors like GlucoWise™, sugarBEAT® and DiaMonTech is reality today. These biosensors are non-invasive and provide real-time lab quality results for glucometry, but, for the purpose of detection of pathogens such devices have not been commercialised yet.This proposal is oriented towards development of cost-effective,

table-top biosensor for instant detection of pathogens, with no antibodies and protien tags invloved. In experiments performed as proof of concept for this proposal, it was found that in presence of perpendicular electric and magnetic fields, the bacteria gyrates [33]. This gyration is attributed to the charge present on the bacterial surface (see Literature Review). The radius and frequency of gyration depends majorly on charge-to-mass ratio of bacteria. Thus, the gyromagnetic ratio can be utilised as a key characterising feature for bacterial strains [33]. Alternating fields alters the physiology of microbial cells [29], beign small, microbes show appreciable response to an external optical signal even at low intensities (see Literature Review). Therefore, analysis of irradiation will help in studying relation between drug-resistance and physiological strength. Motivated by the applications of this project we have marked the objectives as follows

OBJECTIVES

1. **To characterise mass and surface charge of various bacteria (individually and in mixed cultures) and to understand the variation in charge because of cell structure, surface morphology, growth medium and factors like longevity in vitro (LIV) and dilution end point (DEP).**

2. **To study the effect of Electromagnetic (EM) irradiation on pathogens and its implications on physiology, persistence against inhibitory agents and on drug resistance of bacteria.**

LITERATURE REVIEW

Reported methods for rapid detection of bacterial pathogens

Detection and differentiation are two different aspects for assaying of pathogens; detection is generally done after appearance of symptoms on host, while differentiation is to distinctly identify the causative

agent. In 2006, Gaoshan Jing *et al.* designed a micro-fluidic biochip to detect paraffinophilic strains of mycobacterium stained with DAPI [12]. In 2007, Boehm *et al.* measured the variation in electrical impedance due to cell growth in a fixed chamber through platinum electrodes with reported limit of detection (LOD) $9*10^5$ CFU ml^{-1} for *E. coli* cells [13]. In 2007 Cheng *et al.* devised a microfluidic system (with 20 cells/μl LOD) based on change in conductivity of sample after cell lysis [14]. Carbonaro *et al.* developed a micro-fluidic chip with polydimethylsiloxane, having pores in channels, functionalised with proteins for specific interaction with cell surface receptors. Furthermore, successful detection of captured murine erythroleukemia cells was reported on the basis of CD34 surface markers [15]. Radke and Alocilja devised an interdigitated array (IDA) of gold electrodes on silicon substrate, measuring the impedance change due to bacterial cells, *E. coli* and *S. infantis* trapped on the sensor surface through heterobifunctional polyclonal antibody could be detected in 5 minutes [16]. Nucleic acid-based assaying requires cell lysis to extract nucleic acid from pathogens, which is then isolated from other cell contents and purified several times before detection, rigorous purification leads to loss of sample and therefore amplification is must. Yeung *et al.* carried out all the operations including lysis, PCR and detection on a microfluidic device. They utilised magnetic beads coated with capture DNA to extract and purify genomic DNA [17].

Analysis of charge and mass of bacteria

Charge on bacterial surface originates from ionisation of compounds like carboxyl, phosphate and amino groups. Presence of other macromolecules like phospholipids, teichoic acid, teichuronic acid, lipopolysaccharide and various proteins also contribute to cell surface charge. Gram staining techniques greatly utilise surface properties of bacteria, a large number of bacteria can be categorised as gram positive and gram negative based on their cell wall ordonnance. Bacteriologists have been interested in characterisation of bacterial charge, at least since 1920s [18]. A variety of methods have been developed to study cell surface charge, microelectrophoresis (Moyer, 1936) is one of them, but cell adhesion and agglomeration is a major concern in it [19]. Electrostatic interaction chromatography (ESIC) (Wood, 1980) accounts for charge of bacteria on the basis of their adherence to ion-exchange

resin [18]. In 1989, James S. Dickson and Mohammad Koohmaraie studied the surface charge properties for a variety of bacteria to analyse bacterial adhesion to meat surface. Dickson *et. al.* used ESIC along with bacterial adhesion to hydrocarbon (BATH) and concluded surface charge being independent of surface structure [20]. In 1972 Sherbet *et. al*, used isoelectric equilibrium analysis to measure electronegative charge bacterial cells [21]. Ewa Klodzinska *et al.* studied the relation between zeta potential and bacterial behaviour in electrophoretic separation. They reported zeta value of -47mV for *E. coli* cells at pH value 4.99, for *S. aureus* this value was -38 mV under same condition and concentration. The interesting part of their study which will be of crucial importance in our work as well, is the variation in zeta values when two strains were mixed together in different proportions [22]. Thus, it is discernible that mixed strains of bacterial pathogens undergo redistribution of their surface charge.

Unavailability of methods to measure extremely small masses (10^{-12} g) did not let researchers study the mass of bacteria and other microorganisms with subtlety. In 2000s, advances in nanofabrication and its utilisation in CMOS technology opened up ways to fabricate devices like microcantilever arrays, which provided a platform for the measurement and analysis of extremely small masses [23]. M. Loferer *et al.* performed densitometric image analysis to estimate dry weight of *E. coli* and reported that dry mass ranges from 83 fg to 1180 fg [36]. Recently, Daniel Midtvedt *et al.* performed spatio-temporal monitoring of cytosolic mass for *Saccharomyces cerevisiae*. Based on quantitative phase imaging technique, the group reported that mass concentration of cells varies inversely with cell volume [37]. In 2014, Christina *et al.* measured the buoyant masses of live and dead *E. coli* (gram negative) and *Listeria innocua* through commercially available suspended microcantilever resonator Archimedes® and found that mass of live *E. coli* and *L. innocua* were large as compared to their dead counterparts [24]. The density of dead *E. coli* was more as compared to live cells but did not vary in case of *L. innocua.*

From the above discussed facts, it can be stated that mass and charge can be used as a deterministic property for idiosyncratic detection of bacterial pathogens, and can also be used to study various physiological aspects of bacterial cells.

Electromagnetic (EM) characterisation of Bacteria

The relative electrical permittivity ε_r is a substantive electrical parameter that can be determined over a range of frequencies [25]. Methods like microwave cavity perturbation and waveguide transmission can compute electrical conductivity and polarizability of bacterial cultures too, providing an insight to the structural arrangement of charge on bacterial surface. When the input frequency resonates with the natural frequency of constituent molecules the polar molecules lead to absorption of microwave energy, based on this fact, S. Gabriel *et al.* (1996) proposed a theoretical model for microwave characterisation of different tissues including blood, bone and brain etc. [26]. Commercial biosensors based on surface acoustic waves (SAW) like OJ-Bio® are also microwave mediated detection platforms [27]. In 2004, Gaoshan Jing *et al.* developed an out of the league detection method based on microwave cavity perturbation over the frequency range of 10-26 GHz to characterise mycobacteria strains immobilised on paraffin biochip [28]. In 2011, Yuri Shamis *et al.* reported effects of microwave radiation on *E. coli* and reported formation of abnormally larger size pores on the cell surface. The pores were healed soon after the EM field was removed [29]. Dardanoni *et al.* (1988) performed microwave experiments on *Candida albican* using 72 GHz signal modulated at 1KHz and found that 3hours of irradiation reduced the growth rate by 25%. Samarketu *et al*. (1996) performed experiments on *Cyanobacterium* at 9.575 GHz modulated by square wave and observed variation in heterocyst frequency and filament length. Tambiev and co-workers (2001) reported that irradiating 7.1mm wavelength at 2.2mW/cm^2 enhanced the growth rate of *Spirulina platensis* by 50% [30].

Apart from microwave analysis, prolific studies have been performed to examine the effects of other frequencies of EM spectrum like infrared (IR), ultraviolet (UV) and visible light on microorganisms. Soon after 1987, when A. Ashkin and J. M. Dziedzic demonstrated trapping and manipulation of viruses and bacteria through optical lasers, researchers began to explore optical tweezing and its biomedical applications. M. B. Rasmussen *et. al* (2008) investigated the effect of IR on bacterial culture and reported physiological damage on cell surface due to irradiation [34]. UV inactivation of biothreat agents had been a propitious for killing bacteria in food consumables and drinking water, L.J. Rose and H. O' Connell (2008) studied UV-inactivation dosage for different bacteria and found that it

varies for different species [35]. Since the non-thermal effect of EM radiation on biological sample have a complex nature and no single theory could account for all of them. Therefore, it will be an interesting study to electrically scrutinize the competitive behaviour of different bacteria in the same dispersant and to understand how redistribution of bacteria would be reflected in terms of impedance and permittivity when we mix two or more type of strains together.

METHODOLGY

- **Selection of bacterial strains**

  To cover maximum range of diseases that are caused by bacteria, we have selected four different bacterial strains including:

  i. *Escherichia coli (*gram-negative)

  ii. *Salmonella typhimurium (*gram-negative)

  iii. *Listeria monocytogenes* (gram-positive)

  iv. *Staphylococcus aureus (*gram-positive)

  The selected range of pathogens not only covers most of the common diseases but also differs significantly from each other on the basis of their surface morphology like shape (cocci, rod and spiral) and appendages (fimbriae and flagella).

- **Measurement of gyro magnetic ratio of bacterial cell**

  The charge to mass ratio of bacterial cells can be determined by observing the radius and frequency of gyrations for stained bacteria in magneto hydrodynamic (MHD) drift force. The excited mobility of bacteria in response to external electric field can be directly calculated from radius and frequency if applied magnetic field is constant. This mobility (μ_{MHD}) can then be compared with the electrophoretic mobility (μ_e) obtained by performing Zeta potential analysis.

- **Development of Image processing platform**

  MHD based determination of charge requires image processing to analyse frequency and radius. Development of an image processing tool to perform video analysis for direct determination of gyromagnetic ratio are included as a part of this research.

- **Microwave Experiments on bacterial cultures**

  Biological cells absorb microwave energy depending on their molecular composition [26]. Although, bacterial cultures contain large amount of water and watery fluid, but in microwave regime, a small change in concentration of cells lead to significant deviation in permittivity values. Concentrations with varying dilution factor and combination of mixed cultures will be characterised for permittivity, conductivity over the frequency range 1 to 100 GHz.

- **Understanding bacterial physiology through microwave response of bacteria to high frequency (UV, Visible IR) perturbation**

  On different concentrations of bacteria, irradiation of IR, Visible and UV frequencies with varying intensity and exposure time will cause certain physiological damage [34, 35], depending on cell wall strength. Effects of these irradiations, reflected in amended microwave properties will also be analysed in addition to above work.

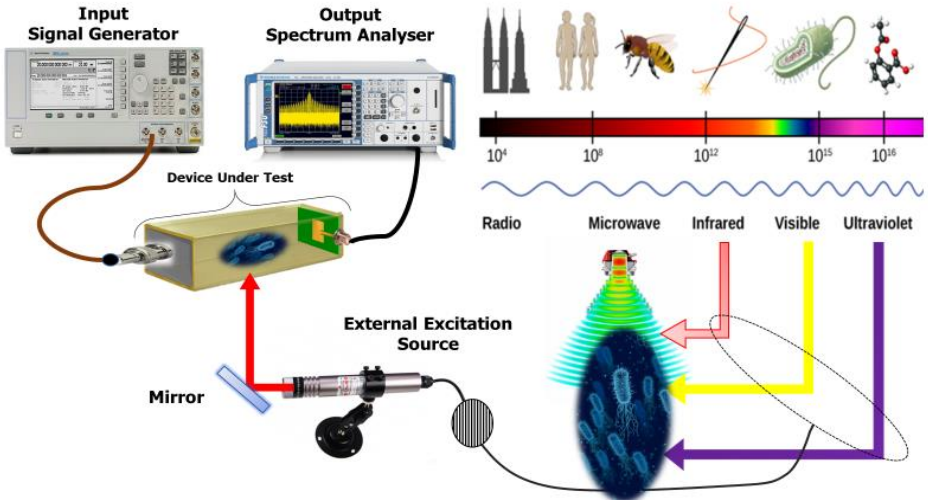
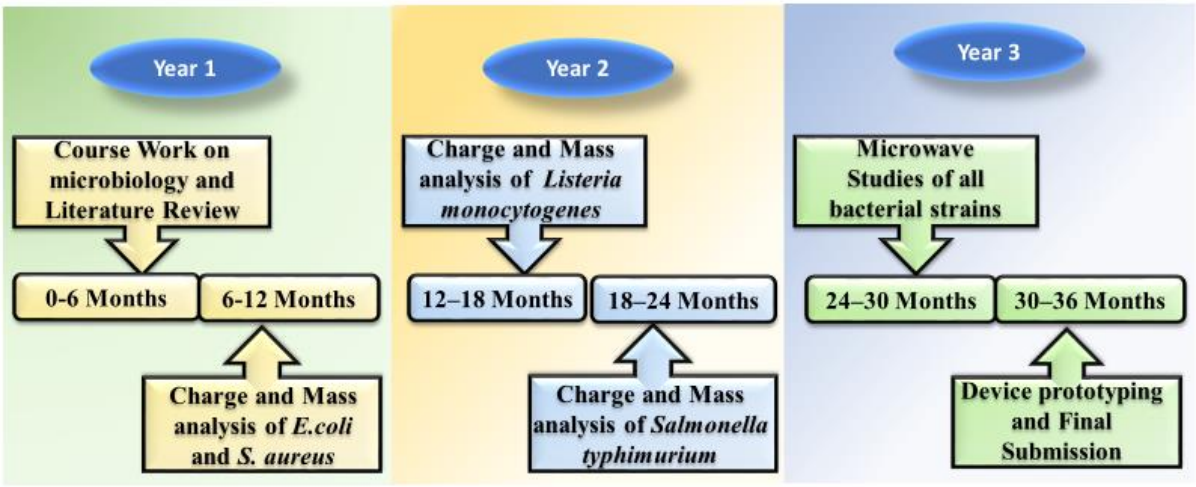


Fig.2 Schematic diagram for microwave characterisation during external irradiation.

Timeline for proposed research work



PROJECTED OUTCOMES

Successful completion of the proposed research may lead to following possible outcomes:

i. **Determination of charge-to mass ratio for different pathogens would lead to development of label free and rapid differentiation methods for bacterial pathogens.**

ii. **Microwave characterisation based on cellular components would help in studying the ability of different pathogens to infect or damage a host (virulence) and will open up ways for specific drug designing and understanding drug-resistance.**

Successful completion of this project will result in development of cost effective and easy-to-use method for pathogen sensing that be used as a point-of-care system in biodefence, animal health care, food safety, pathology and clinical diagnostics.

Presently, each hospitalisation imposes a bill of ₹26,000, which is more than 25% of per-capita income in India [1], [39]. Serological diagnosis for diseases costs several thousand rupees, which increases drastically if the patient is at some remote area. Hence, a tag-free detection platform that can

be used as a table-top sensor in doctor's OPD will help in effectively reducing the detection time and enable timely treatment. As discussed in the beginning, effective management of diseases depends on timely treatment through specific drugs. Therefore, such a sensor may play pivotal role in minimising the nation's economic burden due to diseases and to address the risk of drug resistance through specific differentiation of pathogens. Hence, making a small contribution in building up a happy nation with healthy humans.

***

References

[1] National Health Profile (NHP) of India- 2018:: Central bureau of Health Intelligence http://www.cbhidghs.nic.in/index1.php?lang=1&level=2&sublinkid=88&lid=1138

[2] First Advance Estimates of National Income, 2017-18 released by Central Statistical Office http://timesofindia.indiatimes.com/articleshow/62383138.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst

[3] Sanjay, Thittamaranahalli Varadappa, Gowda Giriyanna, and Shettihalli Gudegowda Kishore. "An evaluation of National Anti-malaria Programme in a district of Karnataka, India." Asian Pacific Journal of Tropical Disease 4 (2014): S595-S598.

[4] Sharma, Devender, and Deepika Sarkar. "Pathophysiology of Tuberculosis: An Update Review." PharmaTutor 6.2 (2018): 15-21.

[5] (Jan, 17) World Health Statistics https: www.who.int/mediacentre/factsheets/fs310/en/

[6] Li, Xiaojing, et al. "Systemic diseases caused by oral infection." Clinical microbiology reviews 13.4 (2000): 547-558.

[7] Levy, Stuart B., and Bonnie Marshall. "Antibacterial resistance worldwide: causes, challenges and responses." Nature medicine 10.12s (2004): S122.

[8] Foster, Timothy J. "The Staphylococcus aureus “superbug”." The Journal of clinical investigation 114.12 (2004): 1693-1696.

[9] Abbasian, Firouz, Ebrahim Ghafar-Zadeh, and Sebastian Magierowski. "Microbiological Sensing Technologies: A Review." Bioengineering 5.1 (2018): 20.

[10] Heo, Jinseok, and Susan Z. Hua. "An overview of recent strategies in pathogen sensing." Sensors 9.6 (2009): 4483-4502.

[11] Mandal, P. K., et al. "Methods for rapid detection of foodborne pathogens: an overview." American Journal Of Food Technology 6.2 (2011): 87-102.

[12] Jing, Gaoshan, et al. "Development of a microfluidic biosensor for detection of environmental mycobacteria." Sensors and Actuators B: Chemical 123.1 (2007): 614-621.

[13] Boehm, Douglas A., Philip A. Gottlieb, and Susan Z. Hua. "On-chip microfluidic biosensor for bacterial detection and identification." Sensors and Actuators B: Chemical 126.2 (2007): 508-514.

[14] Cheng, Xuanhong, et al. "Cell detection and counting through cell lysate impedance spectroscopy in microfluidic devices." Lab on a Chip 7.6 (2007): 746-755.

[15] Carbonaro, Andrea, et al. "Cell characterization using a protein-functionalized pore." Lab on a Chip 8.9 (2008): 1478-1485.

[16] Radke, Stephen M., and Evangelyn C. Alocilja. "A high density microelectrode array biosensor for detection of E. coli O157: H7." Biosensors and Bioelectronics 20.8 (2005): 1662-1667.

[17] Yeung, Siu-Wai, et al. "A DNA biochip for on-the-spot multiplexed pathogen identification." Nucleic acids research 34.18 (2006): e118-e118.

[18] Wilson, W. William, et al. "Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements." Journal of microbiological methods 43.3 (2001): 153-164.

[19] Abramson, Harold A., Laurence S. Moyer, and Andr Voet. "A vertical microelectrophoresis cell with non-polarizable electrodes." Journal of the American Chemical Society 58.12 (1936): 2362-2364.

[20] Dickson, James S., and M. O. H. A. M. M. A. D. Koohmaraie. "Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces." Applied and environmental microbiology 55.4 (1989): 832-836.

[21] Sherbet, G. V., M. S. Lakshmi, and K. V. Rao. "Characterisation of ionogenic groups and estimation of the net negative electric charge on the surface of cells using natural pH gradients." Experimental cell research 70.1 (1972): 113-123.

[22] Kłodzińska, Ewa, et al. "Effect of zeta potential value on bacterial behavior during electrophoretic separation." Electrophoresis 31.9 (2010): 1590-1596.

[23] Alves, Gustavo Marcati A., et al. "Using microcantilever sensors to measure poly (lactic-co-glycolic acid) plasticization by moisture uptake." Polymer Testing 65 (2018): 407-413.

[24] Lewis, Christina L., Caelli C. Craig, and Andre G. Senecal. "Mass and Density Measurements of Live and Dead Gram Negative and Gram Positive Bacteria Populations." Applied and environmental microbiology (2014): AEM-00117.

[25] Oberoi, Sudhi, K. Soami Daya, and Prem Saran Tirumalai. "Microwave sensor for detection of E. coli in water." Sensing Technology (ICST), 2012 Sixth International Conference on. IEEE, 2012.

[26] Gabriel, Sami, R. W. Lau, and Camelia Gabriel. "The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues." Physics in Medicine & Biology 41.11 (1996): 2271.

[27] Palchetti, Ilaria, Peter-Diedrich Hansen, and Damia Barcelo. Past, Present and Future Challenges of Biosensors and Bioanalytical Tools in Analytical Chemistry: A Tribute to Professor Marco Mascini. Vol. 77. Elsevier, 2017.

[28] Jing, Gaoshan, et al. "Developing rapid detection of mycobacteria using microwaves." Analyst 129.10 (2004): 963-969.

[29] Shamis, Yury, et al. "A study of the specific electromagnetic effects of microwave radiation on Escherichia coli." Applied and Environmental Microbiology (2011).

[30] Banik, S. B. A. S. G. S., S. Bandyopadhyay, and S. Ganguly. "Bioeffects of microwave—a brief review." Bioresource technology 87.2 (2003): 155-159.

[31] Lim, Dodge, et al. "Alocilja Magnetic Nanoparticles Efficiently Capture Escherichia coli O157: H7 Isolates." Philippine Journal of Pathology 2.2 (2017): 47.

[32] Weblink for image: http://www.water.me.vccs.edu

[33] Zeeshan, Daya, K. S., Prem Saran Tirumalai, and Evangelyn Alocilja. "Impedance and Magnetohydrodynamic Measurements for Label Free Detection and Differentiation ofE. ColiandS. AureusUsing Magnetic Nanoparticles." *IEEE transactions on nanobioscience* 17.4 (2018): 443-448.

[34] Rasmussen, Mette B., Lene Broeng Oddershede, and H. Siegumfeldt. "Optical tweezers cause physiological damage to Escherichia coli and Listeria bacteria." Appl. Environ. Microbiol. 74.8 (2008): 2441-2446.

[35] Rose, L. J., and H. O'Connell. "UV light inactivation of bacterial biothreat agents." Appl. Environ. Microbiol. 75.9 (2009): 2987-2990.

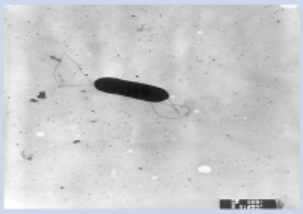
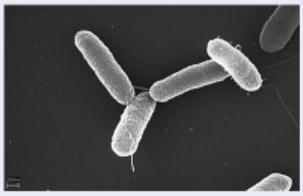
[36] Loferer-Krößbacher, M., J. Klima, and R. Psenner. "Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis." *Appl. Environ. Microbiol.* 64.2 (1998): 688-694.

[37] Midtvedt, Daniel, et al. "Label-free spatio-temporal monitoring of cytosolic mass, osmolarity, and volume in living cells." *Nature communications* 10.1 (2019): 340.

[38] https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death

[39] https://timesofindia.indiatimes.com/business/india-business/indias-per-capita-income-grows-by-8-6-to-rs-1-13-lakh-in-fy18/articleshow/64403580.

APPENDIX

S. No.	Microorganism	Disease Caused	Characteristic Features	Experimental Utility
1	*Escherichia coli*	• Cholecystitis • Bacteraemia • Cholangitis • Urinary tract Infection (UTI) • Bloody Diarrhoea • Other Clinical infections such as neonatal Meningitis and Pneumonia	• Gram-negative • Facultative aerobic • Rod-shaped • Coliform	The rod shape and comparatively high motility, and high electronegative charge.
2	*Staphylococcus aureus*	• Minor skin infections, such as pimples, impetigo, boils and cellulitis etc. • Life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis	• Gram-positive • Cocci-shaped • Arranged in grape-like clusters • Facultative	Non-motile behaviour and clustering would give rise to redistribution of charge and low mobility during electrophoresis.
3	*Listeria monocytogenes* 	• Severe infection in nervous system causing meningitis or encephalitis • Infection of bloodstreams (Sepsis)	• Gram-positive • Firmicutes • Can grow even at 0°C • Rod Shaped • Motile-via-flagella below 30°C	As the bacteria causes problems related to nervous system, charge distribution studies would help understanding the effect due to electrical signal inhibition
4	*Salmonella Typhimurium* 	• Typhoid and paratyphoid fever • Gastroenteritis • Watery Diarrhoea which may be with blood and mucus • Severe vomiting	• Gram-negative • Facultative • Flagellated & Motile • Rod Shaped	The morphology is same as *E. coli* hence charge-to- mass ratio studies would be useful for distinguishing.

***